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(54) Title: METHOD AND APPARATUS FOR DIAGNOSIS

(57) Abstract: A method of diagnosis of a medical condition of a mammal comprising detection of the level of Interleukin-8 (IL-8) in excreted urine of the mammal is described. The use of a detected level of Interleukin-8 in excreted urine of a mammal in the diagnosis of a medical condition of the mammal is also described. The method and use are particularly applicable in the diagnosis of inflammatory conditions such as ulcerative colitis, Crohn's disease, arthritis, cholecystitis, appendicitis, and urinary tract infections. The present invention provides a simple and non-invasive method and apparatus for detecting the level of IL-8 in a mammal, and using the detected level to diagnose, or in the diagnosis of, various medical conditions, particularly but not exclusively inflammatory conditions.

1 "Method and Apparatus for Diagnosis"

2

3 This invention relates to a method and apparatus for
4 diagnosis of medical conditions and in particular to
5 a method and apparatus for non-invasive testing for
6 the diagnosis of inflammatory conditions.

7

8 Inflammatory conditions can be chronic, such as
9 chronic inflammatory bowel disease, chronic
10 arthritis, or acute, such as appendicitis,
11 colecystitis or acute surgical inflammatory
12 conditions. Diagnosis of any inflammatory condition
13 requires a number of very unpleasant and invasive
14 tests. These tests are expensive and can only be
15 performed in hospital by specialists.

16

17 Patients with chronic inflammatory conditions require
18 long term follow up by both primary care physicians
19 and hospital specialists. The natural history of
20 such conditions comprises lull periods and episodes

1 of increased activity. These episodes of increased
2 activity, which may be referred to as 'flare-ups',
3 often require intensive therapy. These episodes
4 requiring more intensive therapy are not always easy
5 to diagnosis. When such an episode is suspected,
6 tests including sigmoidoscopy, colonoscopy, barium
7 enemas, blood tests, radioisotopes, endoscopy, barium
8 meals and histology are ordered. These tests are
9 time consuming, expensive and must be performed by
10 experts, usually in a hospital environment.

11
12 Inflammatory conditions are characterised by the
13 accumulation of inflammatory cells including
14 neutrophils in the inflamed tissue. Neutrophils play
15 a major part in acute mucosa inflammation. For
16 example, in active inflammatory bowel disease
17 neutrophil turnover has been shown to be greatly
18 increased. Neutrophils have a potent ability to
19 produce toxic mediators such as reactive oxygen and
20 nitrogen intermediates. Neutrophils also have a
21 potent ability to produce cytokines.

22
23 Cytokines play a pivotal role in the pathogenesis of
24 inflammation, and some of them have been used in the
25 treatment of certain diseases. Interleukin 8 (IL-8)
26 is a member of the α chemokine family of chemotactic
27 cytokines. It is a potent chemo-attractant and
28 activator of neutrophils. It is present in a wide
29 variety of diseased states. Neutrophils are
30 prominent producers of IL-8 in actively inflamed
31 tissue. That is, tissue damage results from

1 inflammatory cell infiltration. Inflammatory cell
2 infiltration is facilitated by a number of mediators.
3 The chief mediator is IL-8. Thus, patients with
4 active disease have elevated tissue level of IL-8.

5
6 According to one aspect of the present invention,
7 there is provided a method of diagnosis of a medical
8 condition of a mammal comprising detection of the
9 level of Interleukin-8 (IL-8) in excreted urine of
10 the mammal.

11
12 The level of IL-8 in the excreted urine provides a
13 direct indicator of the level of IL-8 in the mammal,
14 and this can be used to diagnose, or be used in the
15 diagnosis of, a medical condition of the mammal.

16
17 Preferably said method is applicable in the diagnosis
18 of inflammatory conditions. Such conditions include
19 at least ulcerative colitis, Crohn's disease,
20 arthritis, cholecystitis, appendicitis, urinary tract
21 infections, etc.

22
23 Said method may have application in the diagnosis of
24 chronic inflammatory conditions, acute inflammatory
25 conditions, and indeed non-specific inflammations and
26 infections.

27
28 According to a second aspect of the present
29 invention, there is provided the use of a detected
30 level of Interleukin-8 (IL-8) in the excreted urine

1 of a mammal in the diagnosis of a medical condition
2 of the mammal.

3
4 According to a third aspect of the present invention,
5 there is provided an apparatus for measuring the
6 level of IL-8 in excreted urine.

7
8 Preferably said apparatus comprises means for
9 immunoassay such as enzyme linked immunosorbent assay
10 (ELISA). Typically said apparatus is provided in kit
11 form. Said apparatus may also be adapted for use in
12 the laboratory. Said apparatus may be adapted for
13 use by a patient.

14 According to another aspect of the present invention,
15 there is provided a method of detecting the level of
16 IL-8 in the excreted urine of a mammal, which method
17 uses measuring means or apparatus as hereinbefore
18 defined, preferably an ELISA assay, wherein urine is
19 added to the means or apparatus and a detection means
20 determines either the or a relative level of IL-8,
21 possibly in comparison with a standard level.

22
23 According to a further aspect of the present
24 invention there is provided a method of diagnosis of
25 a mammal comprising the steps of obtaining a sample
26 of urine excreted by the mammal; testing the level of
27 IL-8 in the urine; and comparing the level obtained
28 against a standard level to determine whether it is
29 significantly abnormal.

30

1 Preferably said method includes the step of testing
2 the level of IL-8 using immunoassay. More preferably
3 said method includes the step of testing the level of
4 IL-8 using enzyme linked immunoassay.

5

6 The present invention, i.e. the measurement of IL-8
7 in urine, can also be used to complement or as a
8 replacement for measuring blood parameters of
9 inflammation like acute phase substances, e.g. C-
10 reactive protein, but with the advantage of being a
11 non-invasive test.

12

13 Embodiments of the present invention will now be
14 described by way of example only with reference to
15 the accompanying drawings in which:

16

17 Fig 1 is a graphic representation of urinary
18 levels of IL-8 in a control group;

19

20 Fig 2 is a graphic comparison of urinary levels
21 of IL-8 in patients with inactive and active
22 ulcerative colitis;

23

24 Fig 3 is a graphic comparison of urinary levels
25 of IL-8 in patients with inactive and active
26 Crohn's disease;

27

28 Fig 4 is a graphic comparison of urinary levels
29 of IL-8 in patients with active and inactive
30 rheumatoid arthritis;

31

1 Fig 5 is a graphic comparison of urinary levels
2 of IL-8 in patients with cholecystitis and a
3 control group;

4
5 Fig 6 is a graphic comparison of urinary levels
6 of IL-8 in patients with appendicitis and a
7 control group;

8
9 Fig 7 is a graphic comparison of urinary levels
10 of IL-8 in patients with urinary tract infection
11 and a control group;

12
13 Fig 8 is a graphic comparison of urinary levels
14 of IL-8 in patients with non-specific
15 inflammation and a control group;

16
17 Fig 9 is a graphic comparison of urinary levels
18 of IL-8 in children with an active
19 inflammation/infection and a control group; and

20
21 Fig 10 is a graphic comparison of urinary levels
22 of IL-8 in children with a viral illness and a
23 control group.

24
25 I have found that the level of IL-8 excreted in urine
26 is a diagnostic indicator of the presence or absence
27 of overall disease activity. This has been confirmed
28 by the following.

29
30 EXPERIMENTAL STUDIES

31

1 Urinary Interleukin-8 Levels in patients with
2 inflammatory bowel disease

3

4 The study involved adult patients who presented at
5 hospital gastroenterology clinics with altered bowel
6 habits, or for further assessment and follow up of
7 previously diagnosed ulcerative colitis; Crohn's
8 disease; or Irritable Bowel Syndrome (IBS).

9

10 The initial diagnosis of chronic inflammatory bowel
11 disease and its activity was based on clinical
12 assessment and from sigmoidoscopic, colonoscopic,
13 histological, and/or barium studies' findings.

14 Clinical assessment included the frequency of
15 diarrhoea stools, bleeding, pain, urgency,
16 haemoglobin, ESR and CRP levels.

17

18 At such clinics, it is routine for patients to give a
19 urine specimen for analysis for protein, blood, sugar
20 etc. For this study, 1ml of each specimen was taken,
21 given a code number, and frozen at -20°C until time
22 of measurement of IL-8. Patients with urinary tract
23 or other systemic infection, renal failure or who
24 were excess consumers of alcohol, cigarettes or
25 diuretics were excluded from the study. Note was
26 taken of any drug therapy for bowel conditions.

27

28

29

30 DEMOGRAPHY

31

	Ulcerative Colitis		Crohn's Disease		Controls
	Active	Inactive	Active	Inactive	
Number	15	19	10	9	14
Males	6	12	3	5	6
Females	9	7	7	4	8
Median age	35	40	32	34	40
Smokers	2	6	4	3	5
Alcohol	8	8	5	3	6

1

2

3 The urine specimens of the above group were tested
 4 for the level of IL-8 present using enzyme linked
 5 immunosorbent assay (ELISA). The specimens were
 6 tested blind. That is, the specimens bore no
 7 indication of any clinical details, or of the basic
 8 diagnosis for the trial participant.

9

10 Full details of the ELISA method of the measurement
 11 of IL-8 are provided hereinafter.

12

13 The raw data of the IL-8 levels obtained is presented
 14 in Table 1 hereinafter and is illustrated graphically
 15 in Figures 1, 2 and 3.

16

17 The results of the assay were then statistically
 18 analyzed. The statistical analysis included multiple
 19 group comparisons using the Kruskal Wallis test,
 20 and, where appropriate, comparison between two

1 groups using the Mann-Whitney test and 95% confidence
2 intervals.
3
4 Results for inflammatory bowel testing
5
6 Kruskall Wallis Test, P = 0.001
7
8 All participants
9 Control Group
10 Median IL-8 level 9.20 pg/ml.
11 Interquartile range 7.8 to 21.1 pg/ml.
12
13 Patients with Active disease
14 Median IL-8 level 75.1 pg/ml.
15 Interquartile range 24.4 to 205 pg/ml.
16
17 Patients with Inactive disease
18 Median IL-8 level 20.1 pg/ml.
19 Interquartile range 10 to 43.1 pg/ml.
20
21 Sub-Groups (by condition)
22 Ulcerative colitis
23 Patients with Active disease
24 Median IL-8 level 104.3 pg/ml
25 Interquartile range 16.1 to 196.0 pg/ml.
26
27 Patients with Inactive disease
28 Median IL-8 level 12.3 pg/ml
29 Interquartile range 6.3 to 26.0 pg/ml.
30
31 Crohn's disease

1 Patients with Active disease
2 Median IL-8 level 53.7 pg/ml
3 Interquartile range 26.3 to 246.6 pg/ml
4
5 Patients with Inactive disease
6 Median IL-8 level 12.0 pg/ml
7 Interquartile range 8.8 to 37.0 pg/ml.
8
9 Mann-Whitney Test
10
11 Control versus Active disease
12 P=0.001
13 95% confidence interval: 15.9, 126.6.
14
15 Control versus Inactive disease
16 P=0.13
17 95% confidence interval: -1.2, 20.5.
18
19 Active disease versus Inactive disease
20 P=0.005
21 95% confidence interval: 8.9, 103.2.
22
23 Active ulcerative colitis versus Inactive ulcerative
24 colitis
25 P =0.002
26 95% confidence interval: 37.5, 127.9.
27
28 Active Crohn's disease versus Inactive Crohn's
29 disease
30 P=0.025
31 95% confidence interval: 6.9, 222.0.

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FURTHER EXPERIMENTAL STUDIES

Urinary Interleukin-8 Levels in Active and Inactive
Rheumatoid Arthritis

A total of 43 patients were studied. Twenty-one had
active arthritis, including 4 men and 17 women, with
a median age of 62 years. Twenty-two patients had
inactive arthritis, including 9 men and 13 women,
with a median age of 56 years.

Interleukin-8 levels in both groups are shown in
Figure 4, and the raw data are presented in the Table
2. Patients with active arthritis have higher levels
compared with those with inactive disease.

Urinary Interleukin-8 Levels in Cholecystitis
Compared with Controls

Fifteen patients with cholecystitis were studied,
including 3 men and 12 women, with a median age of 44
years.

Twenty patients were in the control group, including
6 men and 14 women, with a median age of 48 years.
They presented with non-specific abdominal pain
(n=11) and constipation (n=9).

Interleukin-8 levels in both groups are shown in
Figure 5, and the raw data are presented in Table 3.
Patients with cholecystitis have higher levels of IL-
8 than the controls.

1 Urinary Interleukin-8 Levels in Appendicitis Compared
2 with Controls

3 Eight patients with appendicitis were studied,
4 including 2 men and 6 women, with a median age of 29
5 years.

6 Twenty patients were in the control group, including
7 6 men and 14 women, with a median age of 48 years.

8 They presented with non-specific abdominal pain
9 (n=11) and constipation (n=9).

10 Interleukin-8 levels in both groups are shown in
11 Figure 6, and the raw data are presented in Table 4.
12 Patients with appendicitis have higher levels of IL-8
13 than the controls.

14

15 Urinary Interleukin-8 Levels in Urinary Tract
16 Infection Compared with Controls

17 Twelve patients with urinary tract infection were
18 studied, including 4 men and 8 women, with a median
19 age of 33 years.

20 Twenty patients were in the control group, including
21 6 men and 14 women, with a median age of 48 years.

22 They presented with non-specific abdominal pain
23 (n=11) and constipation (n=9).

24 Interleukin-8 levels in both groups are shown in
25 Figure 7, and the raw data are presented in Table 5.
26 Patients with urinary tract infection have higher
27 levels of IL-8 than the controls.

28

29 Urinary Interleukin-8 Levels in Nonspecific
30 Inflammation Compared with Controls

1 Twelve patients with nonspecific inflammation were
2 studied, including 2 men and 10 women, with a median
3 age of 64 years. They were diagnosed as having
4 diverticulitis (n=8), and gastroenteritis (n=4).

5
6 Twenty patients were in the control group, including
7 6 men and 14 women, with a median age of 48 years.
8 They presented with non-specific abdominal pain
9 (n=11) and constipation (n=9).

10 Interleukin-8 levels in both groups are shown in
11 Figure 8, and the raw data are presented in Table 6.
12 Patients with non-specific inflammation have higher
13 levels of IL-8 than the controls.

14

15 Interleukin-8 in Children (i)

16 Urinary IL-8 Levels in Active Inflammation/ Infection 17 Compared with Controls

18 Routine urine samples were taken from 12 children
19 with active inflammation/infection, including 5 boys
20 and 7 girls, with a median age of 6 years. They were
21 diagnosed as having urinary tract infection (n=7),
22 bacterial meningitis (n=2), tonsillitis (n=1),
23 gastroenteritis (n=1), and appendicular mass (n=1).

24 The control group consisted of 10 children, including
25 a boy and 9 girls, with a median age of 9 years. They
26 were diagnosed as having nonspecific abdominal pain
27 (n=7), diabetes (n=2), and nonspecific headache
28 (n=1).

29 Interleukin-8 levels in both groups are shown in
30 Figure 9, and the raw data are presented in Table 7.

1 Subjects with active inflammation/ infection have
2 higher levels of IL-8 than the controls.

3

4 Interleukin-8 in Children (ii)

5 Urinary IL-8 Levels in Viral Illness Compared with
6 Controls

7 Routine urine samples were taken from 9 children with
8 viral illness, including 5 boys and 4 girls, with a
9 median age of 8 years. They were diagnosed as having
10 viral upper respiratory tract infection.

11 The control group consisted of 10 children, including
12 a boy and 9 girls, with a median age of 9 years. They
13 were diagnosed as having nonspecific abdominal pain
14 (n=7), diabetes (n=2), and nonspecific headache
15 (n=1).

16 Interleukin-8 levels in both groups are shown in
17 Figure 10, and the raw data are presented in Table 8.
18 The levels were similar in the two groups, and no
19 significant differences were found.

20

21

22 ANALYSIS

23

24 From the results of the first study, it can be seen
25 that patients with active inflammatory bowel disease
26 have significantly higher levels of urinary IL-8 than
27 patients with inactive disease. Patients with active
28 inflammatory disease have higher levels of urinary
29 IL-8 than the control group. IL-8 levels in the
30 control group are not statistically different from

1 those in patients with inactive inflammatory bowel
2 disease.

3
4 When the group of patients with active inflammatory
5 bowel disease are sub-divided into those having
6 active ulcerative colitis and those having active
7 Crohn's disease, it is also seen that both groups
8 have higher levels of urinary IL-8 than those in the
9 respective sub-group with inactive disease. This is
10 illustrated in Fig 2 and Fig 3.

11
12 The results of this study show that it is possible to
13 diagnose the likely presence of active inflammatory
14 bowel disease from the urinary level of IL-8.

15
16 From the further studies described hereinbefore, it
17 has been shown that the Interleukin-8 levels in the
18 urine were also elevated in active inflammation in a
19 range of conditions, in both adults and children. In
20 adults, these included:

- 21 - Ulcerative colitis
- 22 - Crohn's disease
- 23 - Rheumatoid arthritis
- 24 - Cholecystitis
- 25 - Appendicitis
- 26
- 27 - Urinary tract infection
- 28 - Nonspecific inflammation/ infection
- 29 (diverticulitis, gastroenteritis).

30

1 Similar results were found in children with active
2 inflammatory processes (with the exception of viral
3 illness causing upper respiratory tract infection).
4 This is because IL-8 is likely to rise in conditions
5 characterised by neutrophilic and macrophage
6 infiltration.

7
8 It is thus clear from the foregoing results that the
9 level of IL-8 in excreted urine is a measure of
10 systemic inflammation. That is, flare-ups in
11 patients known to have e.g. chronic inflammatory
12 bowel disease can be simply and non-invasively
13 diagnosed by determining the level of urinary IL-8
14 for comparison with the normal level of IL-8 for
15 inactive inflammatory disease, or with IL-8 levels in
16 healthy patients.

17
18 This test is comparatively inexpensive, painless and
19 may overtake unnecessary invasive testing or perhaps
20 even unnecessary surgical intervention.

21
22 Patients who are unwell have to suffer a wide variety
23 of unpleasant and invasive tests. Non-invasive
24 testing methods are particularly attractive for use
25 on paediatric patients. Children presenting with any
26 kind of medical condition are usually easily
27 distressed and non-invasive testing ensures that
28 their distress can also be kept to a minimum.

29
30 As previously described this method of diagnosis has
31 application in chronic inflammatory conditions.

1 Examples of such conditions are chronic bowel disease
2 and chronic arthritis.

3
4 In addition, this method of diagnosis has application
5 in acute inflammatory conditions where the level of
6 IL-8 can be compared with the expected level in
7 healthy patients. Examples of such conditions are
8 acute surgical inflammation, appendicitis,
9 colecystitis, pancreatitis and non-specific abdominal
10 pain.

11
12 This method of diagnosis has wide application
13 particularly in paediatric patients for both
14 inflammatory and non-inflammatory conditions. That
15 is, this method of diagnosis can be used in place of
16 blood tests, barium meals, radioisotope tests,
17 endoscopy or colonoscopy, at least in the first
18 instance. The level of IL-8 indicating the presence
19 of active disease or acute inflammation obviously
20 differs in adults and in paediatric patients.

21
22 The results of a test measuring the level of IL-8 in
23 urine are quickly and cheaply obtained using enzyme
24 linked immunosorbent assay. The results have
25 application as a diagnostic tool, or as an adjective
26 aid to diagnosis.

27
28 As a particular example, it is frequently the case
29 that acute appendicitis is mis-diagnosed. That is,
30 the histology of a resected appendix is frequently
31 seen to be normal. The level of the IL-8 in urine is

1 a reliable predictor of presence or absence of intra-
2 abdominal inflammatory process, and is available
3 quickly and cheaply using the above described test.
4 The absence of evidence of intra-abdominal
5 inflammatory process would prevent unnecessary
6 surgical intervention.

7
8 This test is also of use in the self management of
9 chronic inflammatory conditions. That is, an
10 apparatus for measurement of the urinary level of IL-
11 8 will be provided in kit form analogous to a
12 pregnancy test. This will allow patients with known
13 chronic inflammatory conditions to monitor the need
14 for medical intervention. That is, patients
15 diagnosed with chronic inflammatory conditions can
16 confirm a suspicion that their disease may have
17 become active simply by testing a sample of their
18 urine to check if the IL-8 level is significantly
19 high.

20
21 The test could be conducted in hospital laboratories
22 on urinary samples as a matter of routine. The
23 urinary level of IL-8 would give doctors confidence
24 that surgical intervention or increased drug therapy
25 is in fact warranted and necessary.

26
27 The method of diagnosis may be contra-indicated in a
28 small number of conditions including renal failure,
29 or where a patient is known to abuse substances such
30 as alcohol or drugs.

31

1 The present invention provides a simple and non-
 2 invasive method and apparatus for detecting the level
 3 of IL-8 in a mammal, and using the detected level to
 4 diagnose, or in the diagnosis of, various medical
 5 conditions, particularly but not exclusively
 6 inflammatory conditions.

7

8

9 TEST DATA

10

11 Table 1

12

A	B	C	D	E
9.08	196.04	4.4	232.68	11.96
5.4	104.28	9	20.6	7.08
9.24	213.92	3	135.4	36.24
12.8	131.32	11.68	6.56	32.84
48.16	11.12	11.8	49.52	37.76
8.56	1.9	29.84	57.96	11.4
4.12	66.52	29.08	43.2	48.52
18.6	60.44	18.88	567.2	10.6
8.8	138.84	17.4	28.2	3
12.32	219.8	3	288.36	
8.56	16.08	36.68		
232.68	75.12	12.28		
28.64	10.76	3		
4.2	267.16	6.32		
	118.96	20.08		
		10.96		
		43.04		

		19.16		
		25.96		

1

2

3

4

5 Interleukin-8 raw data:

6 A Controls

7 B Active ulcerative colitis

8 C Inactive ulcerative colitis

9 D Active Crohn's Disease

10 E Inactive Crohn's Disease

11

12

13

14 Table 2

15 Active Rheumatoid Arthritis:

16 3 203 70 103 183 13 106 239 227 59 47 93 100 16 17 14

17 51 404 135 311 30

18 Inactive Rheumatoid Arthritis:

19 290 3 10 7 3 5 88 41 7 16 3 68 28 6 24 50 9 138 37 3

20 6 12

21

22

23 Table 3

24 Controls:

25 3 3 22 35 15 3 15 6 3 37 43 11 41 3 3 3 11 10 3 3

26 Cholecystitis:

27 176 48 67 87 321 104 1883 41 184 110 438 379 35 107

28 83

29

1
2 Table 4
3 Controls:
4 3 3 22 35 15 3 15 6 3 37 43 11 41 3 3 3 11 10 3 3
5 Appendicitis:
6 44 272 74 180 319 430 48 44
7
8
9 Table 5
10 Controls:
11 3 3 22 35 15 3 15 6 3 37 43 11 41 3 3 3 11 10 3 3
12 Urinary tract infection:
13 200 1594 551 233 1114 210 616 619 352 1597 343 2641
14
15
16
17 Table 6
18 Controls:
19 3 3 22 35 15 3 15 6 3 37 43 11 41 3 3 3 11 10 3 3
20 Non-specific Inflammation:
21 10 265 187 88 471 328 68 55 568 194 319 57
22
23
24 Table 7
25 Controls:
26 13 15 3 19 5 6 3 7 41 43
27 Active inflammation/infection:
28 204 64 244 423 40 1701 102 1243 61 72 245 193
29
30 Table 8
31 Controls:

1 13 15 3 19 5 6 3 7 41 43

2 Viral illness:

3 9 3 7 5 3 3 12 164 60

4

5 ELISA Method of Measurement for Cytokines

6

7

8 ELISA plates were passively coated with captures

9 antibody overnight at 4°C. mA/b was made up to 1ml

10 (500µg/ml) and 40µl was added to 5.1 ml PBS pH7.2

11 (4µg/ml). This was scaled up as necessary. 50µl was

12 added per well. The plates were shaken to ensure

13 even coverage of wells.

14

15 After O/N incubation at 4°C, the plates were washed

16 once with cold tap water and coating solution was

17 added (100µl per well). They were left for half an

18 hour at room temperature and then flicked out. The

19 plates were air dried and stored at +4°C or at -20°C.

20

21 The ELISA's use paired antibodies. Capture antibody

22 was used to coat the plates, then biotinylated

23 detection antibody was used to detect the bound std.

24 This was quantified by subsequently introducing

25 streptavidin peroxidase, then measured peroxidase by a

26 colour reaction. The plates were washed before use.

27 The standard and samples were added to the wells in a

28 total of 100µl and it was ensured that the standards

29 were diluted in a similar medium to the samples.

30

1 The plates were then sealed using the adhesive plate
2 sealers, and were incubated overnight at 4°C. After
3 the first incubation the plates were washed four
4 times with washing buffer. The stock detection
5 antibody was made up to 1ml (50µg/ml). Then 5µg of
6 stock was added to 6.5 ml assay buffer and used at
7 60µg/well. Again the plates were covered with a
8 plate sealer and incubated for 45 minutes, and were
9 then shaken at room temperature. The plates were
10 then washed four times with wash buffer. Then 100µl
11 of diluted (1:1000) streptavidin (Boehringer) was
12 added to each well at a concentration of 0.2U/ml
13 (100µl of 1:1000 dilution of 200U/ml was used). The
14 streptavidin peroxidase was incubated for 20-30
15 minutes and was shaken at room temperature.
16
17 The plates were again washed four times by wash
18 buffer, then 200µl of substrate was added to each
19 well. The substrate was TMB prepared from stable
20 solutions consisting of:
21 A. Urea-hydrogen peroxide 0.3g per 50ml of 50mM
22 sodium acetate buffer pH 6.0
23 B. Tetramethyl benzidine 2mg/ml in DMF.
24
25 2ml of A and 2ml of B were then added to 20ml of
26 100mM sodium acetate buffer, pH 6.0.
27 The plates were left for approximately 20 minutes
28 until the colour developed (with an aim for a final
29 OD of approximately 1), then 50µl of 2N sulphuric

- 1 acid was added to quench development of colour.
- 2 Measurement was taken within 30 minutes of quenching.
- 3
- 4 ELISA Buffer
- 5 100mM Tris pH 7.2
- 6 150 mg/l 2-methylisothiazolone (Boehringer)
- 7 150 mg/l Bromo nitro dioxane (Boehringer)
- 8 2mg/ml BSA Sigma A3803
- 9 Phenol red 300µl 0.5% solution per litre
- 10 Sodium Chloride 9g/litre
- 11 EDTA 2mM 4ml/litre of 0.5 Molar stock
- 12 Tween 20 0.05% (5ml/l of 10% stock)
- 13 Final pH should be 7.2
- 14
- 15 Preservative:
- 16 Polyvinyl pyrrolidone 2%
- 17 BSA 5mg/ml
- 18 Boehringer preservatives, 0.1% of stock
- 19 EDTAS 5mM

1 CLAIMS

2

3 1. A method of diagnosis of a medical condition of
4 a mammal comprising detection of the level of
5 Interleukin-8 in excreted urine of the mammal.

6

7 2. Use of a detected level of Interleukin-8 in the
8 excreted urine of a mammal in the diagnosis of a
9 medical condition of the mammal.

10

11 3. A method or use as claimed in Claim 1 or Claim 2
12 wherein the medical condition is an inflammatory
13 condition.

14

15 4. A method or use as claimed in Claim 3 wherein
16 the inflammatory condition is a chronic
17 inflammatory condition, an acute inflammatory
18 condition or a non-specific inflammation or
19 infection.

20

21 5. A method or use as claimed in Claim 3 or Claim 4
22 wherein the condition is one of the group
23 selected from ulcerative colitis, Crohn's
24 disease, arthritis, cholecystitis, appendicitis,
25 urinary tract infections.

26

27 6. A method of detecting the level of Interleukin-8
28 in the excreted urine of a mammal wherein urine
29 is added to means for measuring the level of
30 Interleukin-8.

31

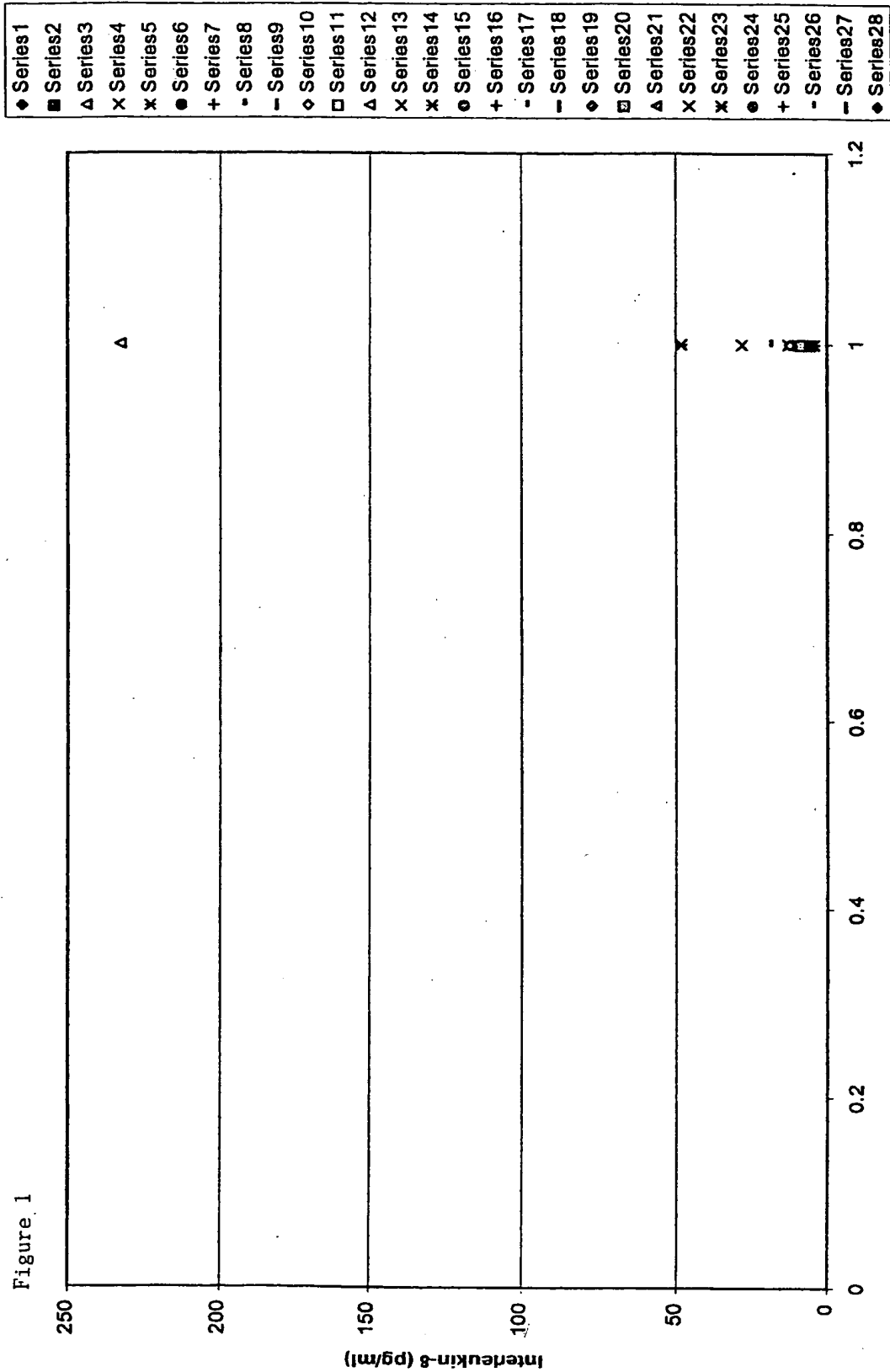
- 1 7. A method as claimed in Claim 6 wherein the
2 measured level of Interleukin-8 is compared with
3 a standard level.
4
- 5 8. A method as claimed in Claim 6 or Claim 7
6 wherein the measuring means is an immunoassay.
7
- 8 9. A method as claimed in Claim 8 wherein the assay
9 is an enzyme-linked immunosorbent assay.
10
- 11 10. A method of diagnosis of a mammal comprising the
12 steps of obtaining a sample of urine excreted by
13 the mammal; testing the level of Interleukin-8
14 in the urine; and comparing the level obtained
15 against a standard level to determine whether it
16 is significantly abnormal.
17
- 18 11. A method as claimed in Claim 10 wherein the
19 method further includes the step of testing the
20 level of Interleukin-8 using immunoassay.
21
- 22 12. A method as claimed in Claim 11 which further
23 includes the step of testing the level of
24 Interleukin-8 using enzyme-linked immunoassay.
25
- 26 13. Apparatus for measuring the level of
27 Interleukin-8 in excreted urine of a mammal.
28
- 29 14. Apparatus as claimed in Claim 13 where the said
30 apparatus comprises means for immunoassay.
31

- 1 15. Apparatus as claimed in Claim 15 wherein the
2 immunoassay is an enzyme-linked immunoassay.
3
- 4 16. Apparatus as claimed in any one of Claims 13-15
5 wherein said apparatus is provided in kit form.
6
- 7 17. Apparatus as claimed in any one of Claims 13-16
8 wherein the apparatus is adapted for use in a
9 laboratory.
10
- 11 18. Apparatus as claimed in any one of Claims 13-17
12 wherein the apparatus is adapted for use by a
13 patient.
14
- 15 19. Apparatus as claimed in any one of Claims 13-18
16 wherein the apparatus is used for measuring the
17 level of Interleukin-8 in excreted urine of a
18 mammal for the diagnosis of an inflammatory
19 condition of the mammal.
20
- 21 20. A non-invasive test for the level of an acute
22 phase substance in the blood of a mammal wherein
23 the level of Interleukin-8 in the urine of the
24 mammal is measured.
25
- 26 21. A test as claimed in Claim 20 wherein the acute
27 phase substance is C-reactive protein.
28
- 29 22. Use of a measurement made by a test as claimed
30 in Claim 20 or Claim 21 in the diagnosis of a
31 medical condition of the mammal.

- 1
2 23. Use as claimed in Claim 22 wherein the medical
3 condition is an inflammatory condition.
4

1/10

Controls



2/10

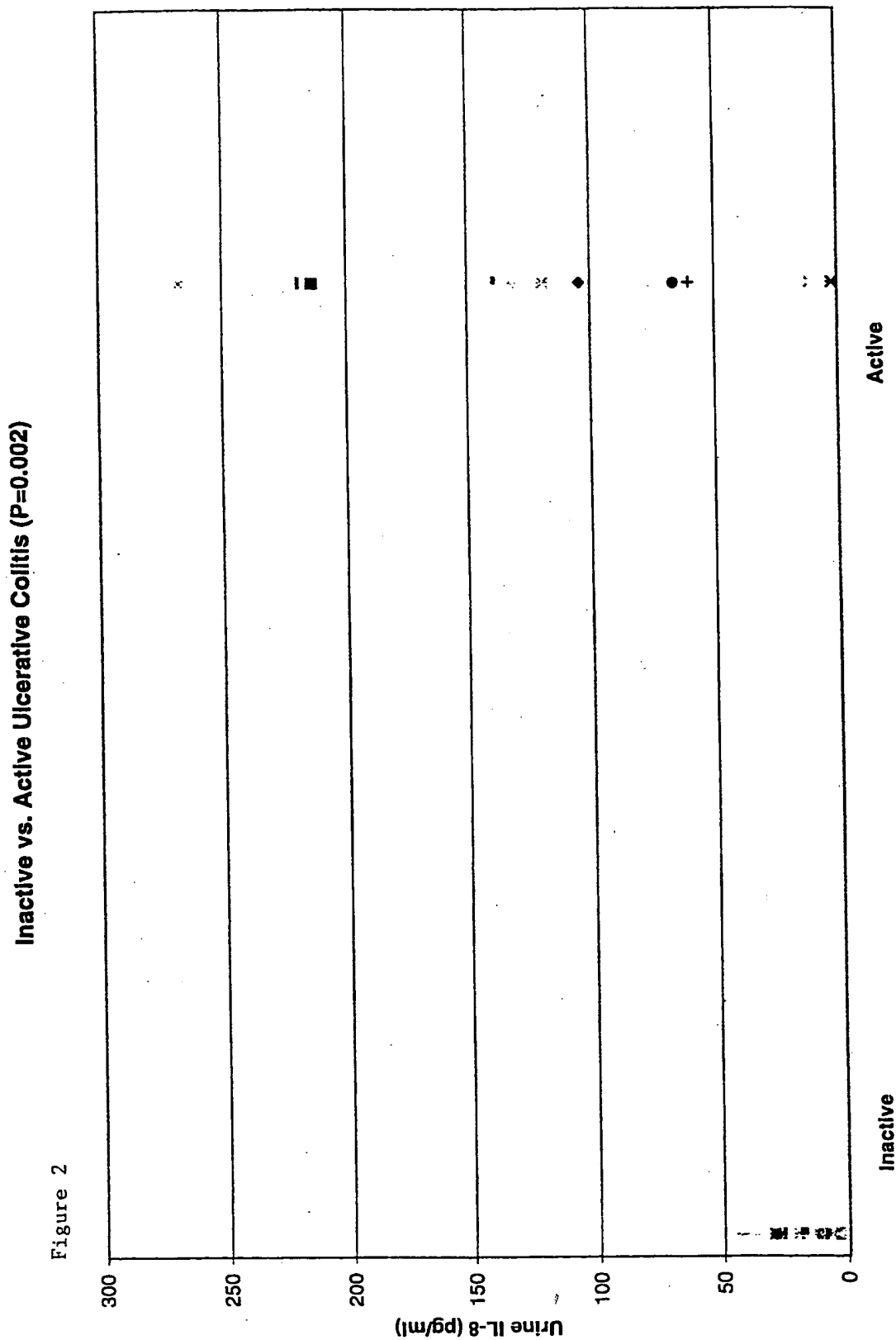
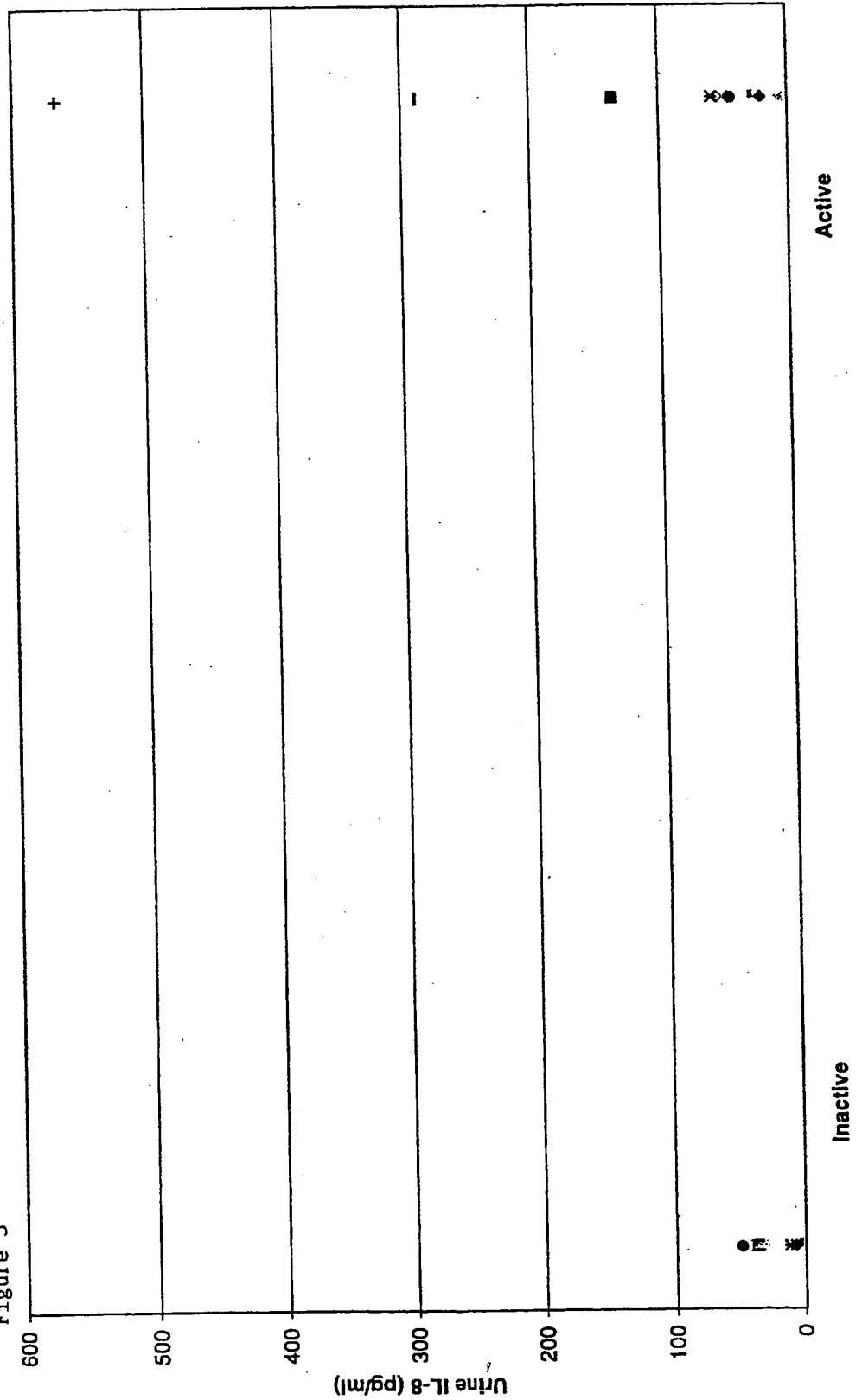


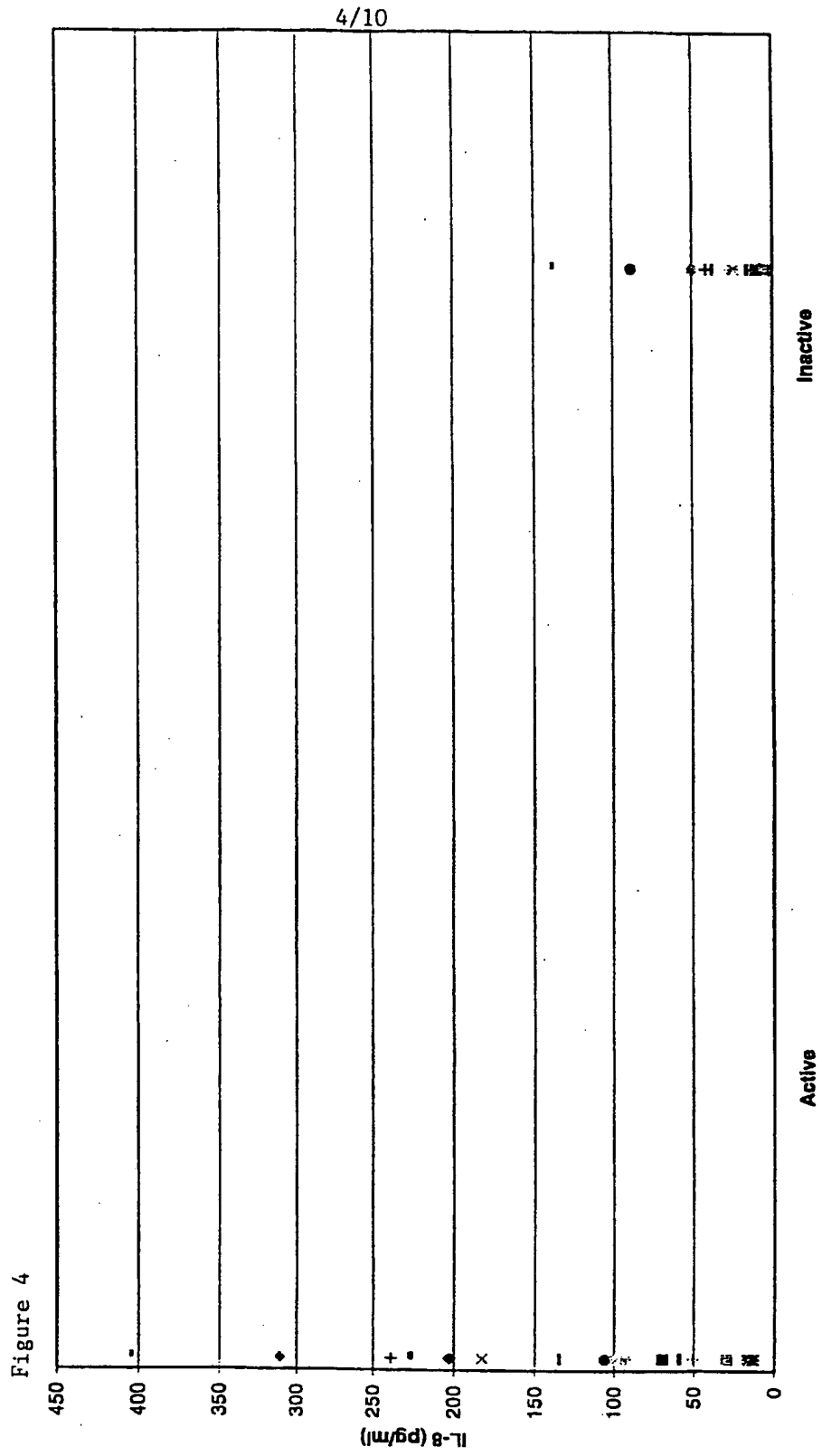
Figure 2

3/10

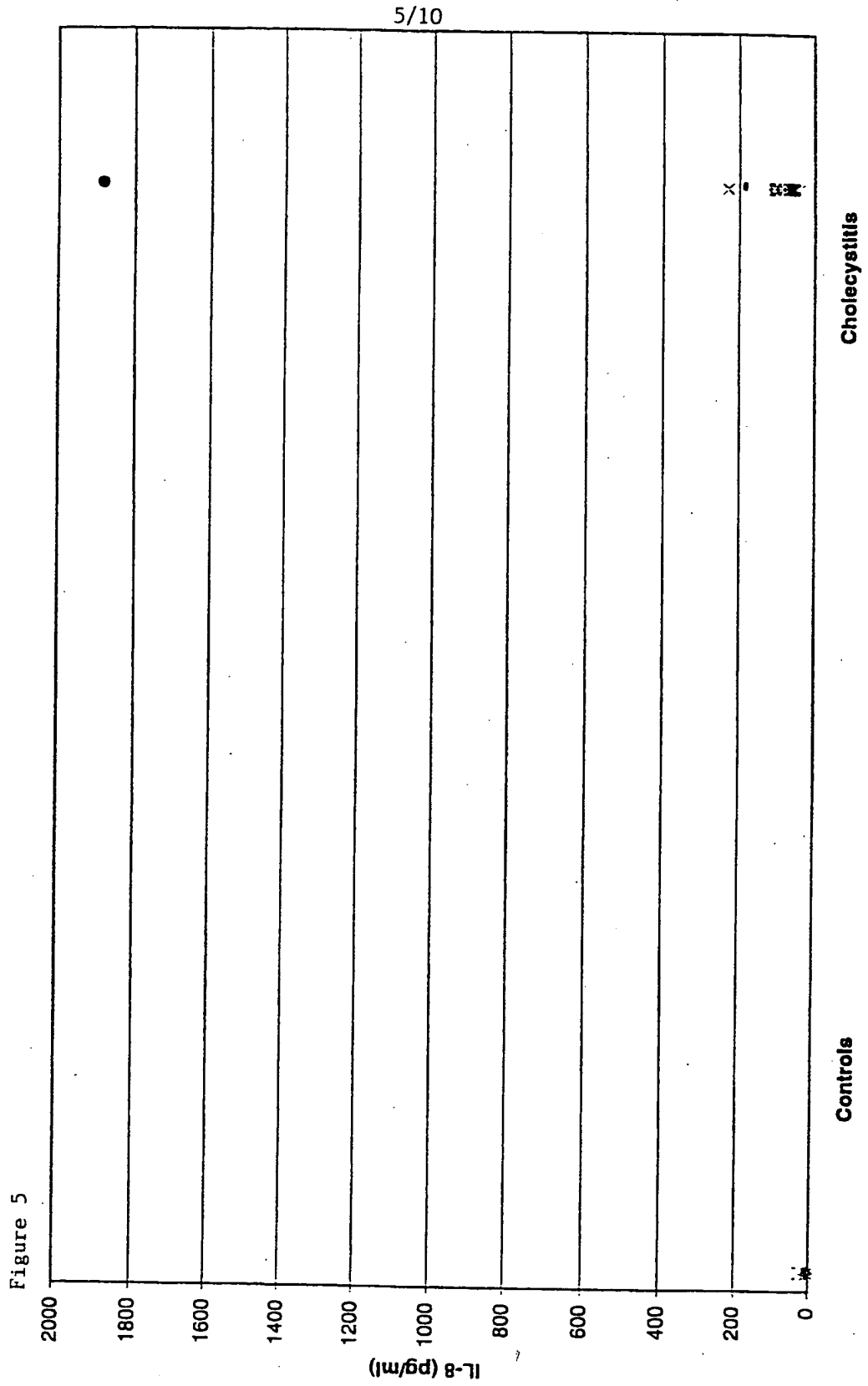
Figure 3
Inactive vs Active Crohn's Disease (P=0.025)



Active vs. Inactive Arthritis (P=0.001)

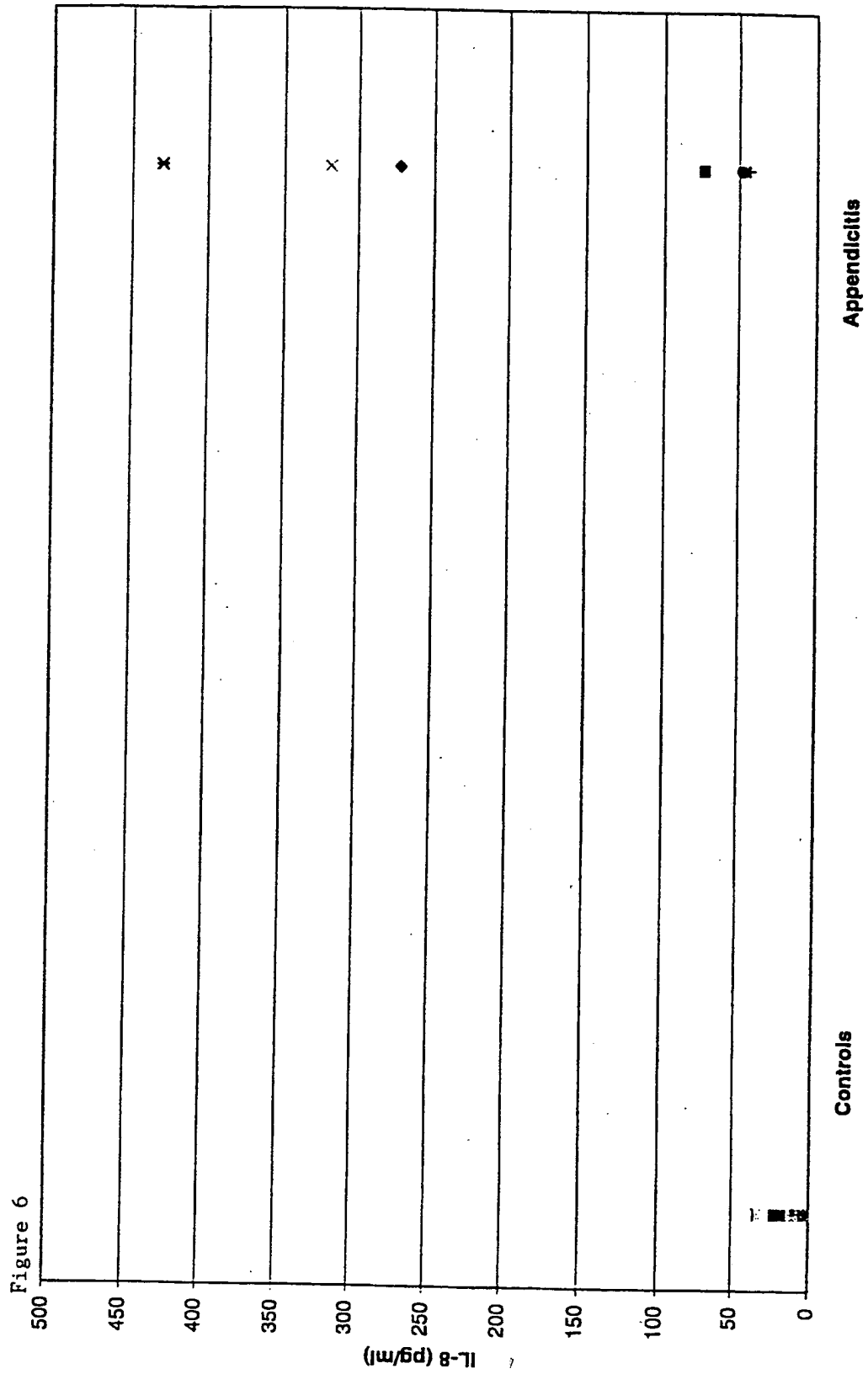


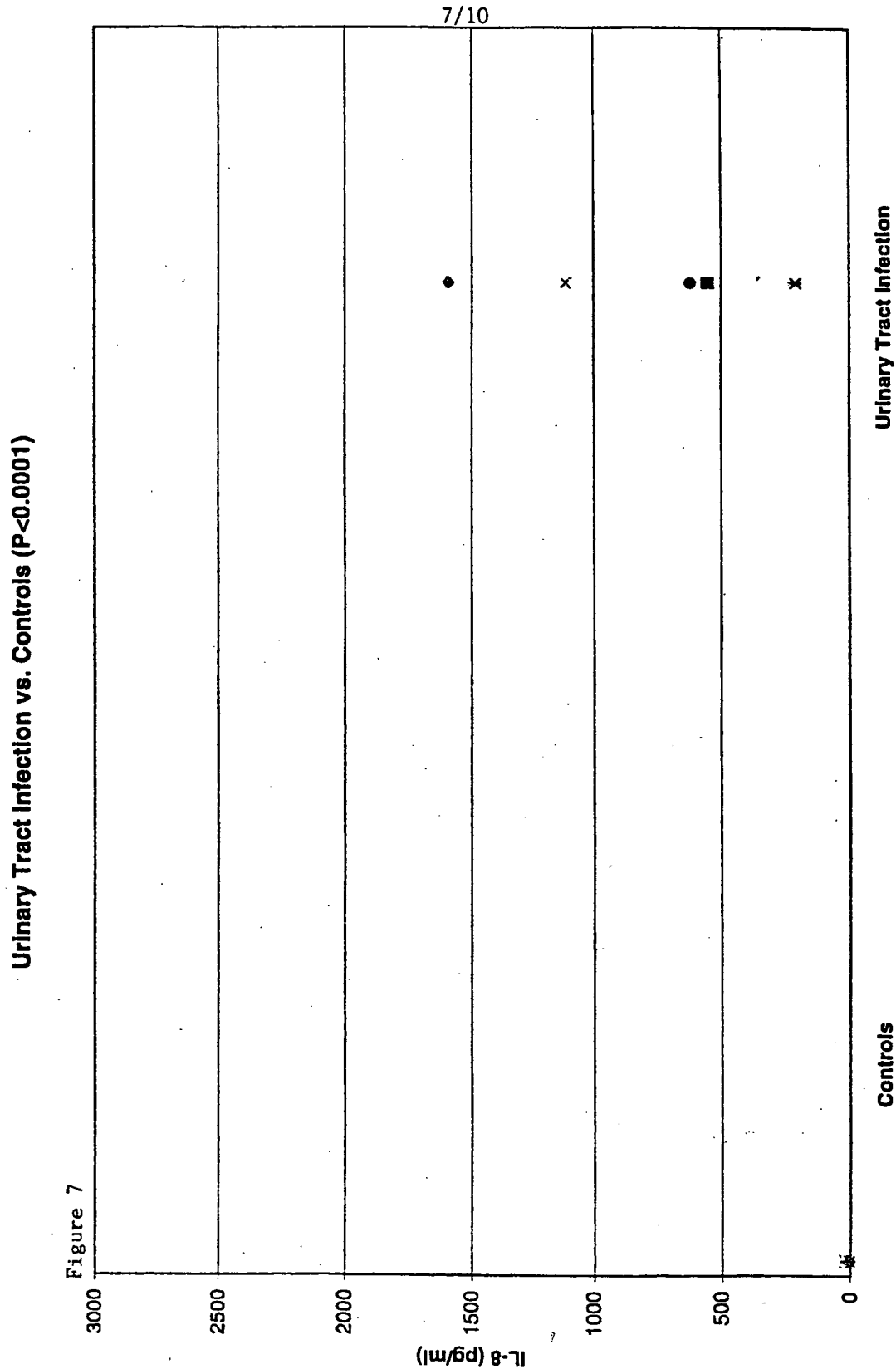
Cholecystitis vs. Controls (P=0.0001)



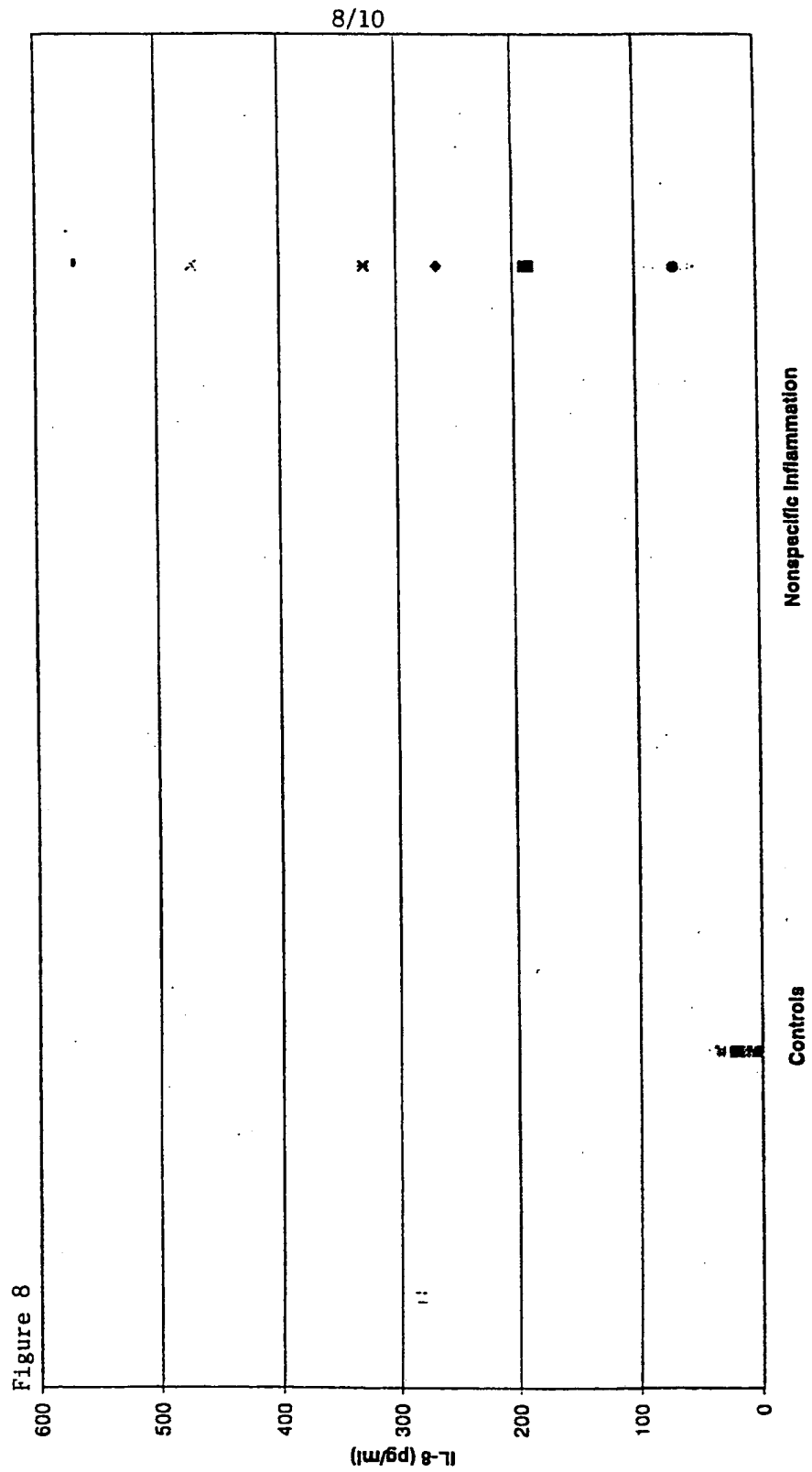
6/10

Appendicitis vs. Controls (P=0.0001)

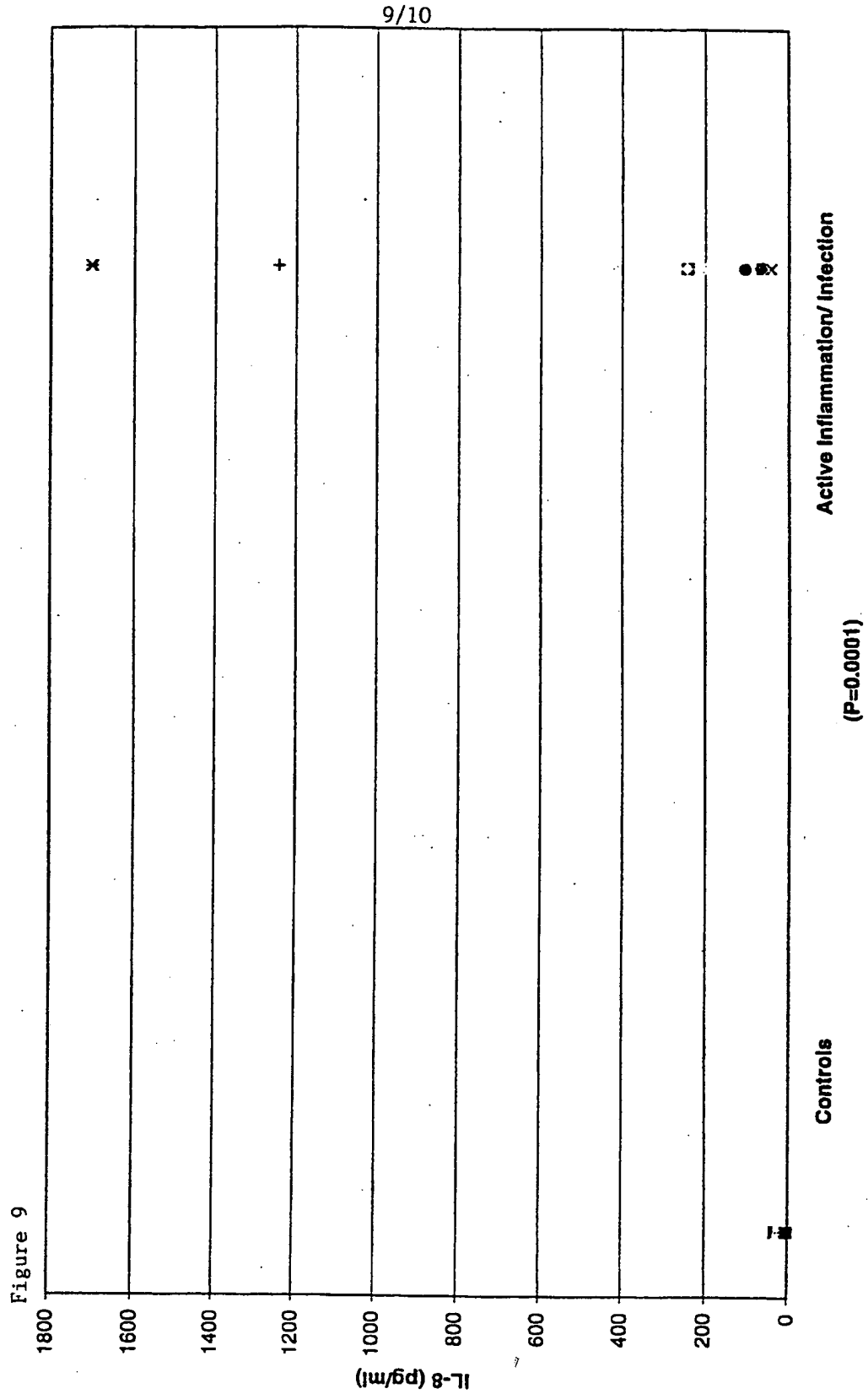




Nonspecific Inflammation vs. Controls (P=0.0001)

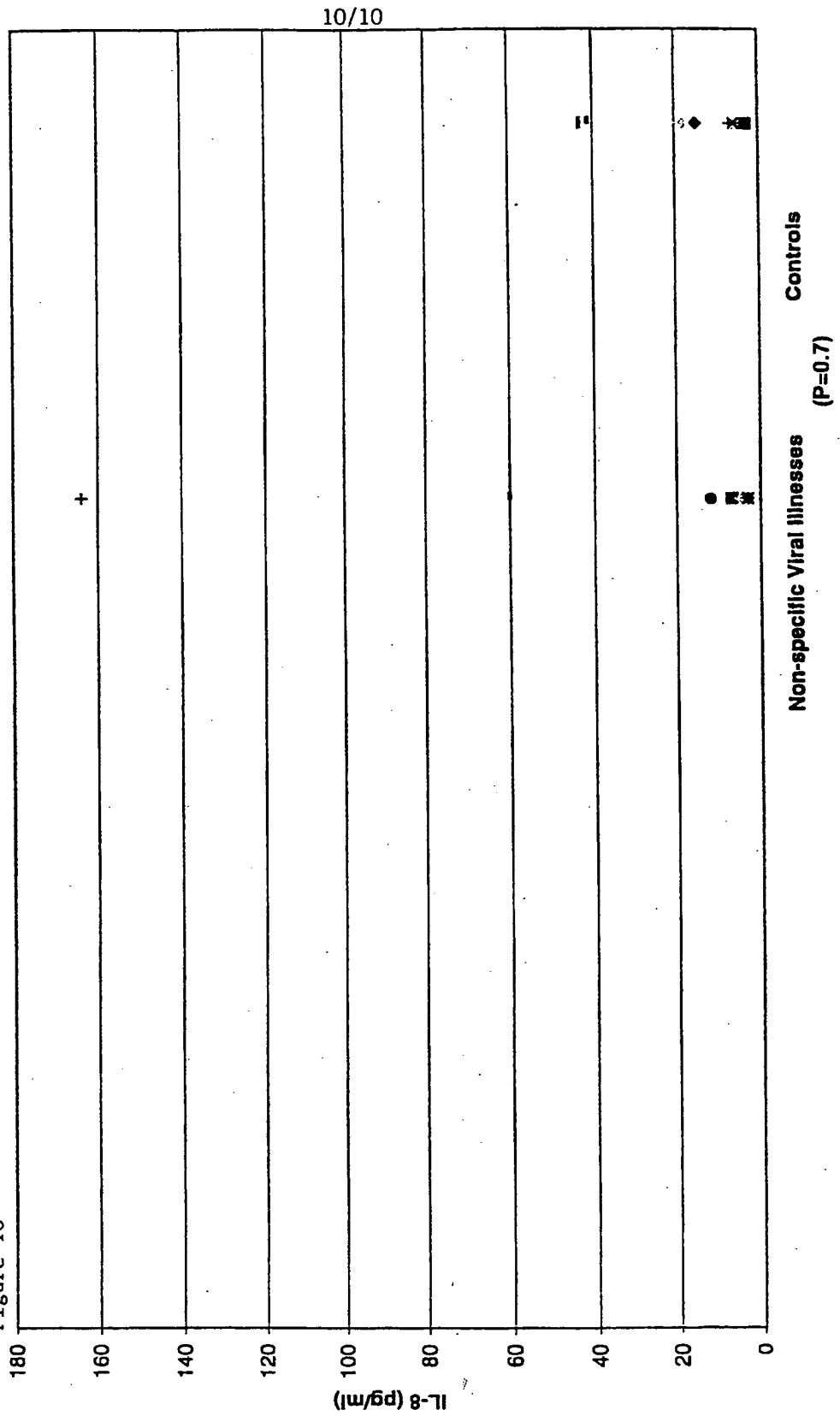


IL-8 in Children



IL-8 in Children

Figure 10



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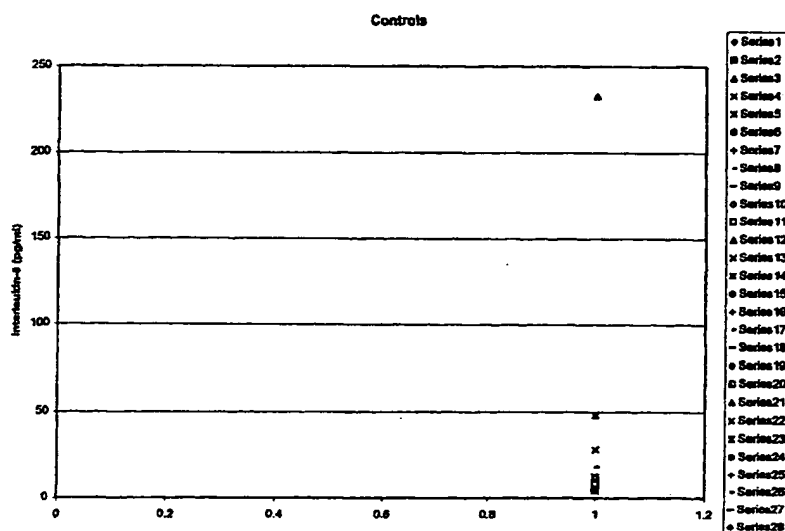
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DIAGNOSIS OF INFLAMMATION MEASURING IL-8 IN URINE



(57) Abstract: A method of diagnosis of a medical condition of a mammal comprising detection of the level of Interleukin-8 (IL-8) in excreted urine of the mammal is described. The use of a detected level of Interleukin-8 in excreted urine of a mammal in the diagnosis of a medical condition of the mammal is also described. The method and use are particularly applicable in the diagnosis of inflammatory conditions such as ulcerative colitis, Crohn's disease, arthritis, cholecystitis, appendicitis, and urinary tract infections. The present invention provides a simple and non-invasive method and apparatus for detecting the level of IL-8 in a mammal, and using the detected level to diagnose, or in the diagnosis of, various medical conditions, particularly but not exclusively inflammatory conditions.

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INTERNATIONAL SEARCH REPORT

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BIOSIS, MEDLINE, EMBASE, CHEM ABS Data, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BENSON MIKAEL ET AL: "Interleukin (IL)-6 and IL-8 in children with febrile urinary tract infection and asymptomatic bacteriuria." JOURNAL OF INFECTIOUS DISEASES, vol. 174, no. 5, 1996, pages 1080-1084, XP000992537 ISSN: 0022-1899 abstract --- -/--	1-23

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STIEMER BEATE ET AL: "Interleukin-8 in urine: A new diagnostic parameter for intra-amniotic infection after premature rupture of the membranes." BRITISH JOURNAL OF OBSTETRICS AND GYNAECOLOGY, vol. 104, no. 4, 1997, pages 499-502, XP000992558 ISSN: 0306-5456 abstract	1-4,6-23
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Y	FLORENT C H: "Inflammatory bowel disease (IBD): Therapeutic actualities." ACTA ENDOSCOPICA, vol. 25, no. 5 SUPPL., 1995, pages 571-574, XP000992520 ISSN: 0240-642X abstract	1,5
Y	---	1,5
Y	TAKAYAMA FUMIO ET AL: "Involvement of interleukin-8 in dialysis-related arthritis." KIDNEY INTERNATIONAL, vol. 53, no. 4, April 1998 (1998-04), pages 1007-1013, XP000992528 ISSN: 0085-2538 abstract	13-19
X	---	1-19
X	JACOBSON, S. ET AL.: "Interleukin-6 and Interleukin-8 in Serum and Urine in Patients with Acute Pyelonephritis in Relation to Bacterial-Virulence-Associated Traits and Renal Function" NEPHRON, vol. 67, no. 2, 1994, pages 172-179, XP000992531 abstract	---
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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TESAR VLADIMIR ET AL: "Cytokines and adhesion molecules in renal vasculitis and lupus nephritis." NEPHROLOGY DIALYSIS TRANSPLANTATION, vol. 13, no. 7, July 1998 (1998-07), pages 1662-1667, XP000992542 ISSN: 0931-0509 abstract; table 2 page 1663, column 2, line 17 - line 28	1-19
X	YASUMOTO RYOJI ET AL: "Urinary interleukin-6 and interleukin-8 in females with urethral syndrome." ACTA UROLOGICA JAPONICA, vol. 43, no. 4, 1997, pages 267-270, XP000992534 ISSN: 0018-1994 abstract	1-19
X	MOUTABARRIK ABDELHAKIM ET AL: "Interleukin-8 in patients with glomerulonephritis." JN JOURNAL OF NEPHROLOGY, vol. 8, no. 6, 1995, pages 325-330, XP000992536 ISSN: 1121-8428 abstract	1-19
X	TIKHONOV I ET AL: "A study of interleukin-8 and defensins in urine and plasma of patients with pyelonephritis and glomerulonephritis." NEPHROLOGY DIALYSIS TRANSPLANTATION, vol. 12, no. 12, December 1997 (1997-12), pages 2557-2561, XP000992540 ISSN: 0931-0509 abstract	1-19
X	WO 96 41194 A (CHILDRENS MEDICAL CENTER) 19 December 1996 (1996-12-19) claims 1,2,5	1-19

Information on patent family members

PCT/GB 00/02971

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